OPINION

Iron–sulphur cluster biogenesis and mitochondrial iron homeostasis

Tracey A. Rouault and Wing-Hang Tong

Abstract | Iron–sulphur clusters are important cofactors for proteins that are involved in many cellular processes, including electron transport, enzymatic catalysis and regulation. The enzymes that catalyse the formation of iron–sulphur clusters are widely conserved from bacteria to humans. Recent studies in model systems and humans reveal that iron–sulphur proteins have important roles in mitochondrial iron homeostasis and in the pathogenesis of the human disease Friedreich ataxia.

Iron–sulphur (Fe–S) clusters are indispensable participants in three processes that are central to life on earth — respiration, photosynthesis and nitrogen fixation. These ancient modular cofactors consist of iron atoms that are directly coordinated to inorganic sulphide or the cysteinyl sulphurs of the associated proteins. Fe–S clusters are biologically versatile and can function in a wide variety of crucial electron transfer and biosynthetic processes. In addition, Fe–S proteins have important regulatory functions. Work in both yeast and mammalian systems supports the conclusion that defects in the enzymes involved in Fe–S cluster assembly lead to significant mitochondrial iron overload and failure. However, the mechanism by which the compromise of Fe–S cluster synthesis leads to mitochondrial iron overload is unknown.

Here, we discuss important features of Fe–S chemistry and biology, with emphasis on the potential importance of Fe–S proteins in the regulation of mammalian cellular and mitochondrial iron metabolism. Fe–S proteins function as important regulatory proteins in systems ranging from bacteria to mammals. We propose that mitochondrial iron homeostasis might be a highly regulated process in which an Fe–S protein functions as a sensor for the mitochondrial iron status or aids in the formation of such a sensor. An improved understanding of the relationship between Fe–S cluster synthesis and mitochondrial iron overload is important in understanding the pathogenesis of the human disease Friedreich ataxia.

Fe–S proteins in eukaryotes

Fe–S proteins are an ancient, ubiquitous and functionally diverse class of metalloproteins that are found in organisms from bacteria to humans[1,2]. The unique features of these remarkably versatile cofactors enable Fe–S proteins to transfer electrons, catalyse enzymatic reactions, and function as regulatory proteins (FIG. 1). The underlying architectural element of the Fe–S cluster is the [2Fe–2S] rhomb that is formed by two tetrahedrally coordinated iron atoms with two bridging sulphides, and complexes that contain up to eight iron atoms can be conceptualized as an elaboration of the basic [2Fe–2S] unit (FIG. 1a). The combination of the chemical reactivity of iron and sulphur, together with variations of cluster composition, oxidation, spin states and the local protein environment, enables Fe–S clusters to function in numerous distinct biological roles. Known mammalian Fe–S proteins and their functions are listed in TABLE 1, but this list is probably far from complete, not only because of the limitations of sequence-based methods for predicting potential Fe–S proteins, but also because Fe–S clusters often spontaneously disassemble during aerobic purification.

Fe–S proteins have a wide range of reduction potentials (–700 mV to 400 mV; REF. 3) and are among the most important electron carriers in nature. They are integral components of respiratory and photosynthetic electron transfer chains, in which groups of Fe–S clusters function as a linear series of redox centres or electron reservoirs[4,5]. Up to 12 different Fe–S clusters can be found in the

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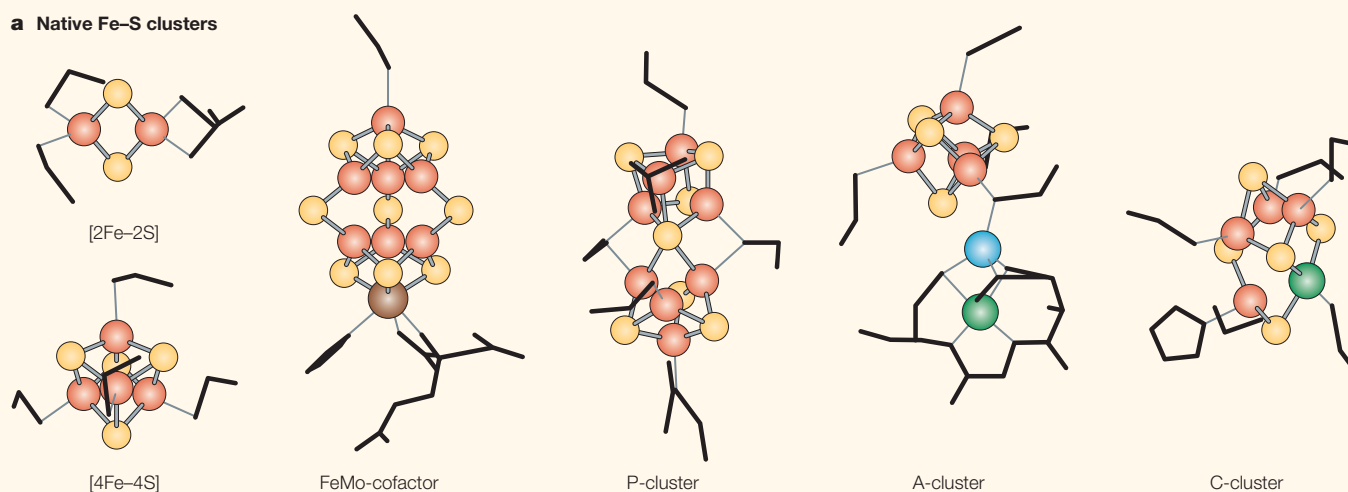
mitochondrial electron transport chain[4]. In chloroplasts, Fe–S clusters are required for the function of photosystem I, ferredoxin and the cytochrome $b_6 f$ complex[5].

The ability of Fe–S clusters to coordinate ligands also allows them to facilitate various enzymatic functions[2,3,6]. Mitochondrial aconitase is part of the citric acid cycle, which provides free energy for ATP generation. In aconitase, a single iron atom of the [4Fe–4S] cluster facilitates the dehydration–hydration reaction that reversibly converts citrate to isocitrate by ligating the hydroxyl group of the substrate and activating this group for elimination (FIG. 1b). The sulphur atoms of Fe–S clusters can also be involved in catalysis (FIG. 1b). Fe–S clusters also provide some enzymes with structural stability. The Fe–S clusters in several DNA repair enzymes, including the endonuclease III homologue NTH1 (REF. 7) and the MutY/MYH family of DNA glycosylases[8], are required for the recognition and repair of DNA damage.

The sensitivity of Fe–S clusters towards species such as H_2O_2, NO, and O_2^- also allows them to function as important regulatory sensors that respond to oxidative stress and intracellular iron levels in bacteria[9] and mammals[10] (FIG. 1c). Mammalian cells encode two aconitases: the mitochondrial enzyme (mentioned above) that functions in the citric acid cycle, and a cytosolic enzyme, iron regulatory protein-1 (IRP1), that is bifunctional[11]. In its [4Fe–4S] cluster form, IRP1 functions as an aconitase. When the Fe–S cluster is absent, for example, as a result of Fe depletion or the presence of reactive oxygen species, IRP1 binds mRNAs that contain a specific stem–loop sequence that is known as the iron-responsive element (IRE). When IRP1 binds to the 5′ untranslated region of ferritin mRNA, the translation of ferritin — an iron storage protein — is repressed. When IRP1 binds to the 3′ untranslated region of transferrin receptor mRNA, which produces an iron-uptake protein, the transcript is protected from degradation. As a result, IRP1 binding to mRNAs can decrease iron sequestration and increase cellular iron uptake. However, the Fe–S cluster of IRP1 is stable at the low oxygen concentrations in mammalian cells[12], and IRP1 is not a key contributor to iron regulation in healthy animals[11,13]. Nevertheless, IRP1 can be induced to bind mRNA by stimuli such as H_2O_2, NO or O_2^- (REF. 14), which indicates that it might be important in pathophysiology.

Mechanism of Fe–S cluster assembly

As early as the 1970s, studies showed that Fe–S clusters can be synthesized *in vitro*[1], but it was not until the late 1980s that pioneering work

by Dean and co-workers identified the first enzymes that catalyse Fe–S cluster formation in bacteria[15]. To date, at least three operons — *nif, isc* and *suf* — have been shown to encode genes that are involved in bacterial Fe–S cluster biosynthesis (for a review, see REF. 15). The *nif*-specific genes in *Azotobacter vinelandii* are involved in the assembly of Fe–S clusters in nitrogenase. *Escherichia coli* contains the *isc* and *suf* gene clusters that are involved in the general maturation of Fe–S proteins. Homologues of the bacterial *isc* genes have been identified in yeast, plants and animals[15,16], and homologues of the bacterial *suf* genes are found in cyanobacteria and plants[16–18].

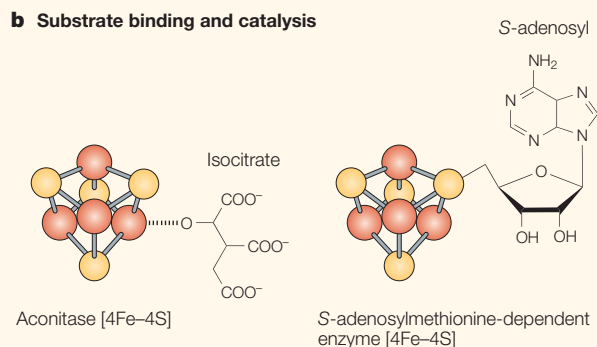Most of the insights into the mechanism of Fe–S cluster assembly have come from the extensive genetic and biochemical studies that have been carried out in *E. coli* and in *Saccharomyces cerevisiae*[15,19,20] (FIG. 2). IscS is a pyridoxal-phosphate-dependent cysteine desulphurase. Nucleophilic attack on the cysteine sulphur of the substrate, by an active site cysteine residue in IscS, results in the formation of an enzyme-bound persulphide (S–IscS). Sulphur transfer and iron acquisition leads to assembly of [2Fe–2S] and [4Fe–4S] clusters on the scaffold proteins IscU, Nfu and IscA. Cluster assembly requires the reduction of the persulphide sulphur, and redox proteins such as ferredoxin and glutaredoxin might be required to complete cluster formation. IscU is a substrate for the chaperone proteins HscA/HscB, which might assist the unfolding and refolding of scaffold proteins during cluster assembly, or might function in the release of the Fe–S cluster during its transfer to the target protein (FIG. 2). Studies in cyanobacteria[17,21], plants[18,22] and animals[23–25] support the idea that the basic assembly mechanisms are conserved across species. So, because of the sequence and functional similarities, whenever possible in following sections, we apply the nomenclature that was originally proposed for the *E. coli isc* operon to the mammalian Fe–S cluster assembly proteins and genes. In bacteria, Fe–S cluster assembly takes place in the cytoplasm, whereas in mammals and plants, Fe–S cluster assembly takes place in several compartments (discussed later).
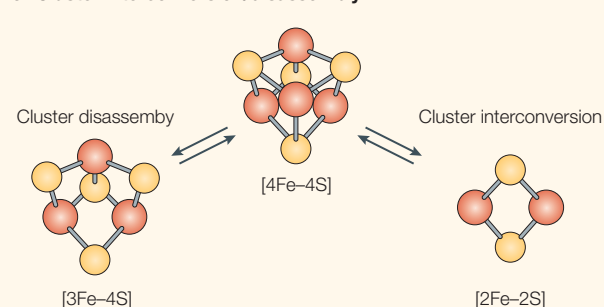

Figure 1 | **The structural and chemical versatility of iron–sulphur (Fe–S) clusters. a** | The Fe–S cluster types that are most common are the [2Fe–2S] and [4Fe–4S] clusters. More complex Fe–S clusters are found in the active site of nitrogenase (the FeMo-cofactor and P-clusters) and in carbon monoxide dehydrogenase (A- and C-clusters), and these clusters can be thought of as an elaboration of these basic units. The clusters shown are colour coded by atom type: iron, red; sulphur, yellow; molybdenum (Mo), brown; nickel, green; and copper, blue. Protein ligands are shown in black. **b** | Examples of substrate binding and catalysis in relation to the iron-based and sulphur-based chemistry of Fe–S clusters. In aconitase, the substrate isocitrate/citrate (isocitrate is shown) is activated when its hydroxyl group becomes ligated to one of the irons of the [4Fe–4S] cluster of aconitase. In *S*-adenosylmethionine-dependent Fe–S enzymes, the adenosylation of a bridging sulphur in a [4Fe–4S] cluster might be directly involved in the mechanism of enzymatic activity (for further information about this process, see REF. 6). **c** | Fe–S clusters have a remarkable capability for structure interconversion, ligand-exchange reactions and oxidative degradation. Reactive oxygen species including superoxide (O_2^-) and nitric oxide (NO) can oxidize clusters, which leads to cluster interconversion or disassembly, as well as to conformational and activity changes in the Fe–S regulatory proteins that sense intracellular Fe, O_2, O_2^- and NO. Although there are well-characterized examples of both [4Fe–4S]–[3Fe–4S] and [4Fe–4S]–[2Fe–2S] interconversion, there is no known example of interconversion between all three of the structures shown. Part **a** was modified with permission from REF. 69 © (2003) American Association for the Advancement of Science .

Mitochondrial iron homeostasis

Recent genetic studies in *S. cerevisiae* show that, in addition to providing Fe–S clusters for many different metabolic pathways, Fe–S cluster biogenesis is crucial for maintaining mitochondrial iron homeostasis. Yeast strains that were depleted of the cysteine desulphurase Nfs1, the homologue of IscS, had a >30-fold increase in mitochondrial iron levels compared with wild-type strains[26,27] (FIG. 3a). Similarly, yeast strains that were depleted of Fe–S cluster scaffold proteins (Isu1, Isu2, Isa1, Isa2 and Nfu1), chaperone proteins (Ssq1 and Jac1) or ferredoxin (Yah1) all showed a marked accumulation of iron in their mitochondria, as well as mitochondrial oxidative damage[26–31]. Yeast strains with mutations in *NFS1* and *SSQ1* increased their transcription of the iron-uptake system through the activation of the iron-sensing transcription factor Aft1 (REF. 27). Recent studies indicate that Aft1 might not directly sense elemental iron, but instead might sense Fe–S clusters or a signal from an Fe–S protein[32,33].

Abnormal Fe–S protein biogenesis and mitochondrial iron accumulation are prominent in the heart and central nervous system of patients with Friedreich ataxia[34,35]. This condition is an autosomal recessive, cardiac and neurodegenerative disorder that is commonly caused by a decreased expression of the frataxin transcript, owing to the expansion of a GAA triplet repeat in the first intron of the frataxin gene[34–36]. In the brains and hearts of patients with Friedreich ataxia, the enzymatic activities of mitochondrial Fe–S proteins decrease and iron accumulates in the mitochondria[37] (FIG. 3b).

Although the exact function of frataxin remains unclear, many studies indicate that frataxin participates in Fe–S cluster biogenesis. Depletion of the yeast frataxin homologue, Yfh1, leads to severe defects in respiration, the loss of Fe–S enzyme activities, mitochondrial
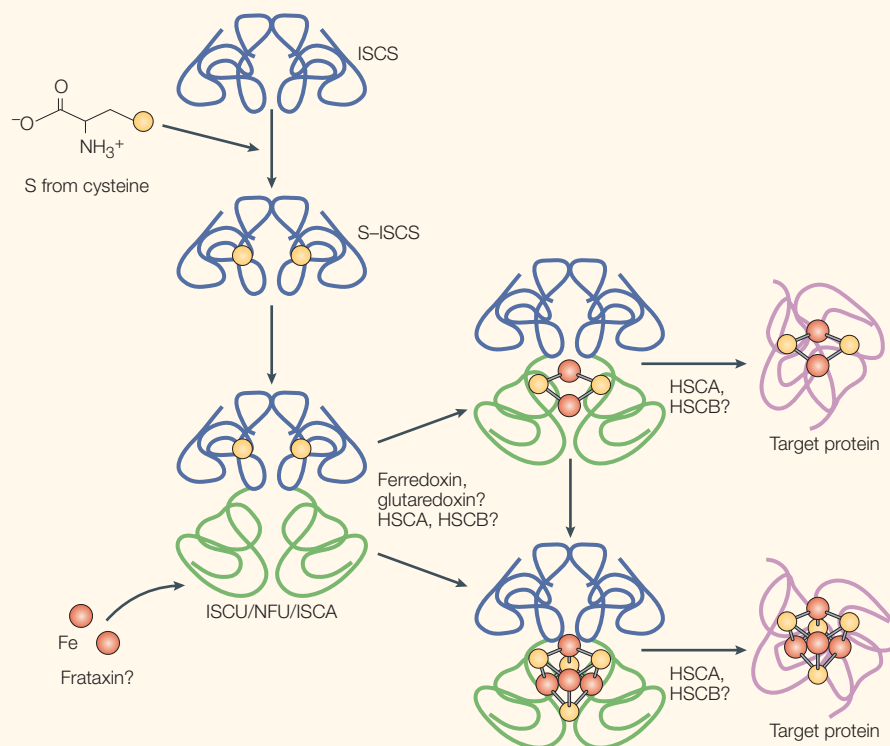
Figure 2 | **A proposed mechanism for iron–sulphur (Fe–S) cluster biosynthesis.** This shows a proposed pathway for the assembly of Fe–S clusters. This process takes place both in mitochondria and in the cytosol of mammalian cells. Because of the sequence and functional similarities, mammalian Fe–S cluster assembly proteins are shown here using the nomenclature that was originally proposed for the *Escherichia coli isc* operon. The cysteine desulphurase ISCS provides the sulphur (S; yellow circles) for cluster formation on scaffold proteins (ISCU, NFU and ISCA). The chaperone proteins HSCA and HSCB might facilitate cluster maturation and transfer the cluster to the target protein, and the redox proteins ferredoxin and glutaredoxin might provide the reducing equivalents that are necessary for Fe acquisition and cluster assembly[15]. Frataxin might be involved in delivering iron for Fe–S cluster biogenesis.

iron overload (FIG. 3a), the loss of mitochondrial DNA, and hypersensitivity to oxidative stress[35,38]. Depletion of Yfh1 also results in a decrease in the *de novo* synthesis of Fe–S clusters on Isu1 (REF. 20), and glutathione S-transferase pulldown experiments and a synthetic lethal screen have provided biochemical and genetic evidence for an interaction

between Yfh1, Nfs1 and Isu1 (REFS 39,40). Furthermore, frataxin can deliver iron to rebuild damaged forms of the Fe–S cluster in mammalian mitochondrial aconitase[41]. Conditional mouse models for Friedreich ataxia indicate that the disruption of mitochondrial Fe–S cluster assembly precedes the development of mitochondrial iron overload[42,43] (FIG. 3c). Human and yeast frataxin can oligomerize and sequester iron[44], which led to the idea that frataxin might function in mitochondrial iron storage and detoxification[45]. However, this hypothesis cannot explain why the reintroduction of *YFH1* into *YFH1*-deficient yeast results in increased iron export from mitochondria to the cytosol[46]. In addition, recent site-directed mutagenesis studies show that Yfh1 is functional *in vivo* without oligomerization[47]. Taken together, these results imply that frataxin is involved in delivering iron for Fe–S cluster biogenesis and that the compromise of Fe–S biogenesis in Friedreich ataxia leads to mitochondrial iron overload, which, in turn, leads to mitochondrial oxidative damage and failure.

Table 1 | **Mammalian iron–sulphur proteins**

Iron–sulphur protein	Function	Subcellular location
Respiratory complexes I–III	Electron transport chain	Mitochondrial inner membrane
Mitochondrial aconitase	Citric acid cycle	Mitochondrial matrix
Ferrochelatase	Haem biosynthesis	Mitochondrial matrix
NTH1/MYH*	DNA repair	Mitochondrial matrix, nucleus
RpS3	Protein synthesis, DNA repair	Cytosol, nucleus
IRP1/cytosolic aconitase	Iron metabolism	Cytosol
Xanthine oxidase	Purine metabolism	Cytosol
Phosphoribosylpyrophosphate amidotransferase	Purine biosynthesis	Cytosol

*NTH1 is the mammalian homologue of *Escherichia coli* endonuclease III, and MYH is the mammlian homologue of the *E. coli* DNA glycosylase MutY. IRP1, iron regulatory protein-1; RpS3, ribosomal protein S3.

Why does the disruption of Fe–S cluster assembly lead to mitochondrial iron overload? One explanation is that an Fe–S protein might either function as a sensor for mitochondrial iron status or aid in the function of such a sensor. As both iron overload and iron deficiency can cause cell death, mammals have evolved highly specialized mechanisms in the cytosol to control the import, export and distribution of reactive iron[48]. However, the components and mechanisms that are involved in maintaining mitochondrial iron homeostasis are not well understood (FIG. 4). Fe–S clusters are key to the sensing and regulatory functions of several transcription factors in bacteria, including FNR, SoxR, IscR and SufR, as well as in the mammalian cytosolic regulator IRP1 (REFS 9,11). If an Fe–S protein is directly or indirectly involved in the sensing and regulation of mitochondrial iron homeostasis, the decreased formation of this Fe–S protein in the mitochondria could be registered by the cell as signalling mitochondrial iron deficiency. This might result in feedback regulation and the activation of a response that

includes the increased delivery of iron to mitochondria and decreased mitochondrial iron export. Cytosolic iron pools might become functionally depleted because of mitochondrial sequestration, as has been observed in some mutant yeast strains that are depleted of Fe–S cluster assembly proteins[27,29,32]. The misregulation of mitochondrial iron homoeostasis and cytosolic iron depletion might engage the cell in a vicious cycle in which increased cellular iron uptake further exacerbates mitochondrial iron overload[27,49].
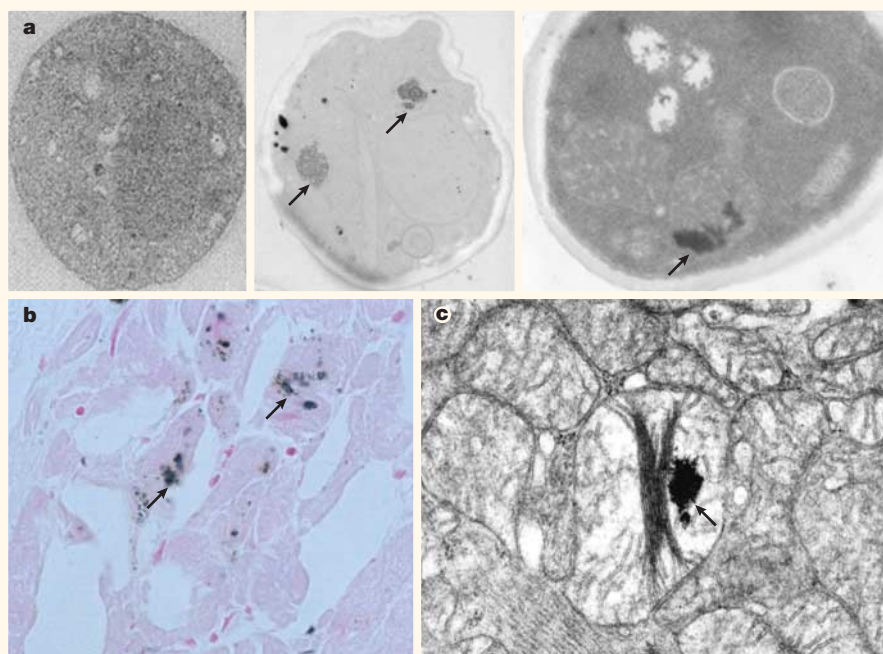
An alternative hypothesis is that iron exits the mitochondria in the form of Fe–S clusters, and that it remains trapped in mitochondria when the assembly of Fe–S clusters is disrupted or if the function of an Fe–S cluster exporter is compromised[50,51]. In humans, X-linked hereditary sideroblastic anaemia is caused by mutations in the human mitochondrial inner membrane ATP-binding cassette transporter ABC7 and is associated with massive mitochondrial iron overload[52,53]. Deletion of yeast ATM1, which encodes a homologue of ABC7, leads to mitochondrial iron accumulation and

Figure 3 | Mammalian iron overload and human diseases. Mitochondrial iron overload is found in yeast that have compromised iron–sulphur (Fe–S) cluster synthesis, as well as in the hearts and brains of patients with Friedreich ataxia and mouse models. **a** | Normal yeast cells do not contain electron-dense material in their mitochondria (left). However, iron staining and electron microscopy (EM) showed that yeast with a missense mutation in the *NFS1* gene for a cysteine desulphurase (middle) or a deletion in the *YFH1* gene for the yeast frataxin homologue (right) are loaded with iron (arrows). Images courtesy of Andrew Dancis, University of Pennsylvania, Philadelphia, USA. **b** | Ferric iron was detected by Prussian blue staining in heart muscle from a patient with Friedreich ataxia (arrows). Image courtesy of Massimo Pandolfo, Université Libre de Bruxelles, Brussels, Belgium. **c** | EM analysis of the heart muscle of a conditional mouse model of Friedreich ataxia highlights the deposition of electron-dense iron in a mitochondrion in which the cristae have collapsed. Image courtesy of Helene Puccio, Centre National de la Recherche Scientifique/Institut National de la Santé et de la Recherche Médicale/Université Louis Pasteur, Illkirch, France.

decreased iron binding in two putative cytosolic Fe–S proteins[26,54]. It was proposed that ABC7/Atm1 exports preformed Fe–S clusters from mitochondria into the cytosol, and that iron remains trapped in mitochondria when the assembly or export of Fe–S clusters is impaired[50]. However, the overexpression of an Atm1 homologue, Mdl1, can partially compensate for *ATM1* loss (Mdl1 reduces the mitochondrial iron level in Atm1-deficient cells by 50%), and Mdl1 has previously been shown to function in the export of peptides out of mitochondrial matrix[55]. The exact function of ABC7/Atm1 therefore remains unclear[33].

In yeast, the disruption of Fe–S cluster biogenesis and mitochondrial iron metabolism are linked to extensive changes in nuclear gene expression and to changes in other subcellular compartments. Deletion of the yeast frataxin homologue increases the expression of >70 genes, including many that are involved in cellular iron uptake and homeostasis[49]. *MRS3* and *MRS4* were initially proposed to encode mitochondrial iron transporters, but more recent studies indicate that the deletion of these mitochondrial proteins also alters cellular iron uptake and vacuolar metal homeostasis[56]. It seems that the maintenance of iron homeostasis in different subcellular compartments is interlinked, although the precise signals and connections between the various compartments are unclear.

The process by which nuclear gene expression alters in response to changes in mitochondrial iron status has been termed mitochondrial retrograde regulation, and is an important process in normal and pathophysiological conditions[57]. Communication between mitochondria and the nucleus is required for the activation of the mitochondrial stress response. The release of peptides that are derived from the degradation of mitochondrial proteins has been proposed to be involved in the mitochondrial stress signalling pathway[58]. By analogy, it is possible that the sensing of

mitochondrial iron status involves the release of signal peptides from the mitochondria through Atm1 or Mdl1. Interestingly, deletion of the gene that encodes the mitochondrial GTP/GDP exchanger, *YHM1*, also leads to the misregulation of the cellular iron uptake systems and the accumulation of mitochondrial iron. This indicates that changes in mitochondrial GTP levels might be another signal that is important for mitochondrial iron homeostasis[59]. It is possible that mutations in *ATM1*, *MDL1*, *MRS3*, *MRS4* or *YHM1* alter mitochondrial iron homeostasis indirectly by altering the generation or transport of signal molecules that regulate mitochondrial iron uptake, efflux and distribution (FIG. 4). Determining the nature of the signals and the connections will be key to understanding the regulation of mitochondrial iron homeostasis.

Genetic studies in yeast have contributed substantially to our current view of mitochondrial iron homeostasis. However, it is important to note that there are fundamental differences between iron metabolism in yeast and in mammalian cells[48,60]. The mammalian cytosolic iron regulators IRP1 and IRP2 are not present in yeast. In the cytosol of mammalian cells, the assembly and disassembly of a [4Fe–4S] cluster in IRP1 and the iron-dependent degradation of IRP2 provide the mechanisms for sensing intracellular iron levels. In the tissues of healthy animals, most IRP1 contains a [4Fe–4S] cluster and functions as a cytosolic aconitase. Its homologue, IRP2, is therefore responsible for regulating the levels of the transferrin receptor and ferritin, as well as probably one isoform of the iron importer divalent metal transporter-1 and the iron exporter ferroportin, by binding to iron-responsive elements in their mRNAs[48] (FIG. 4). By contrast, in yeast, the transcription factors Aft1 and Aft2 regulate the expression of genes that are necessary for iron uptake, and whether Aft1 or Aft2 directly bind iron and/or Fe–S clusters is still under debate[32,33]. Unlike mammalian cells, yeast do not have cytosolic or mitochondrial ferritin, instead they store excess iron in the vacuole. It is therefore important to consider these differences when studies in yeast are used as a reference for understanding mammalian mitochondrial iron homeostasis.

Evolution of Fe–S cluster biogenesis

Interest in the process of Fe–S cluster assembly has grown in recent years, not only because of the medical relevance of this process, but also because species comparisons offer a potential way to trace eukaryotic history[61]. In yeast, with the exception of Nfs1, Cfd1 (REF. 62) and Nar1 (REF. 54), Fe–S cluster

assembly proteins have so far been detected only in mitochondria[28,30,31,51]. Nfs1 (IscS) is present in mitochondria[26] and in the nucleus[63,64], and is important not only for Fe–S cluster assembly, but also for the thio-modification of mitochondrial and cytosolic tRNAs[64]. Cfd1 and Nar1 are present in the cytosol and are proposed to be involved in the delivery/insertion of Fe–S clusters into cytosolic targets[54,62]. The recent detection of
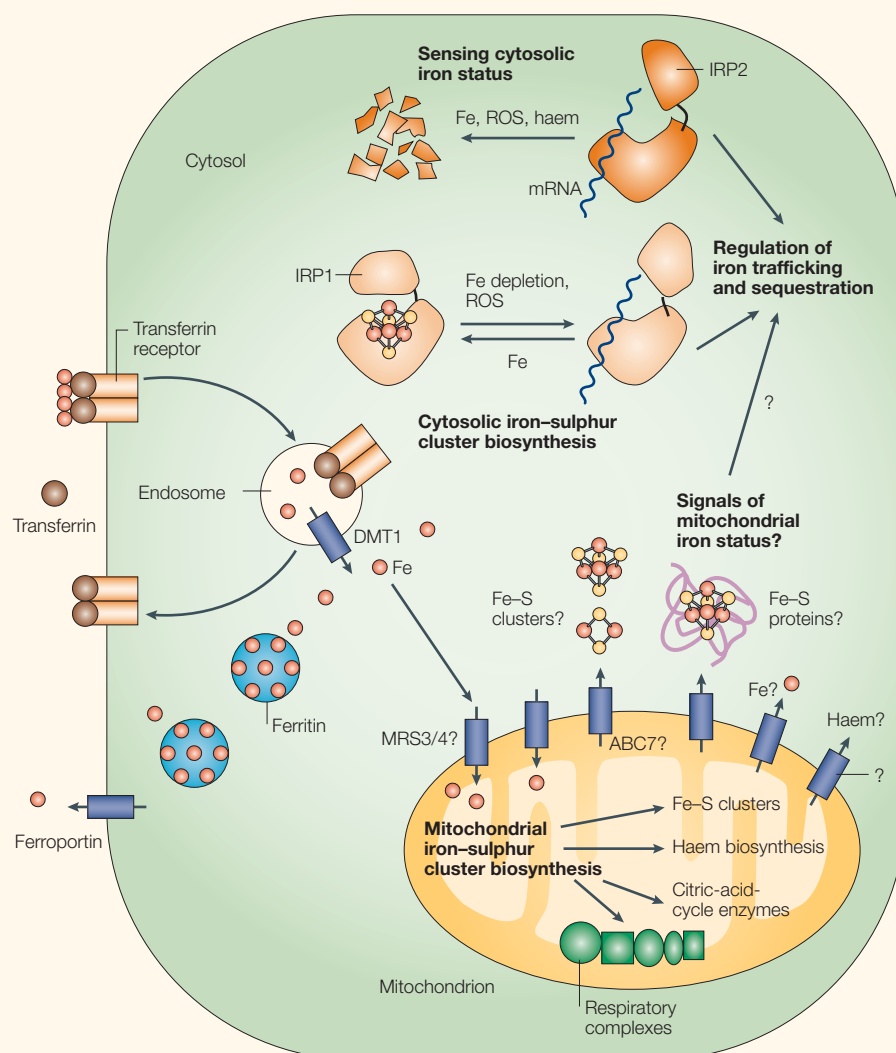
Figure 4 | **Iron–sulphur (Fe–S) cluster biogenesis in mammalian iron homeostasis.** Extracellular diferric transferrin proteins are bound by the dimeric transferrin receptor and internalized by receptor-mediated endocytosis. Iron is then transported into the cytosol through divalent metal transporter-1 (DMT1). In the cytosol of mammalian cells, the assembly and disassembly of a [4Fe–4S] cluster in iron regulatory protein-1 (IRP1) and the iron-dependent degradation of IRP2 provide the mechanisms for sensing intracellular iron levels. In the tissues of healthy animals, most IRP1 contains a [4Fe–4S] cluster and functions as a cytosolic aconitase. Its homologue, IRP2, is therefore responsible for regulating the levels of the transferrin receptor and the iron storage protein ferritin (which can store up to 4,000 Fe atoms per molecule), as well as probably one isoform of DMT1 and the iron exporter ferroportin, by binding to iron-responsive elements in their mRNAs[48]. IRP1 might contribute to iron regulation in pathophysiological situations. Mitochondrial Fe–S protein biosynthesis seems to require the proteins ISCS, ISCU, NFU, ISCA, HSCA, HSCB, ferredoxin, glutaredoxin and frataxin (not shown; see FIG. 2). ISCS, ISCU and NFU might also function in Fe–S cluster assembly in the cytosol (not shown). The mitochondrial transporters that are involved in iron uptake and in the efflux of Fe, Fe–S clusters, Fe–S cluster proteins and haem groups (ferrochelatase is an Fe–S enzyme that is involved in haem biosynthesis in mitochondria; see TABLE 1) have only been partially characterized[26,70], and the mechanism of sensing and regulation remains unclear. However, genetic studies in *Saccharomyces cerevisiae* indicate that the sensor/regulator of mitochondrial homeostasis is an Fe–S protein or that it senses an Fe–S-cluster-containing protein[32,50]. Please note that because of the sequence and functional similarities, mammalian Fe–S cluster assembly proteins are mentioned here using the nomenclature that was originally proposed for the *Escherichia coli isc* operon. ABC, ATP-binding cassette; ROS, reactive oxygen species.

homologues of IscS and IscU in the mito-chondrial remnant organelles (mitosomes) of *Giardia intestinalis*[61] (an intestinal protozoan pathogen), as well as a homologue of IscS in *Trichomonas vaginalis* hydrogenosomes (which are thought to have evolved from the same proteobacterial ancestor as mitochon-dria)[65], supports the idea that the essential metabolic functions of Fe–S proteins might have provided selection pressure for the permanent establishment of mitochondrial endosymbionts in early eukaryotic cells.

Comparative genomics and proteomics studies from the most primitive parasitic cells to metazoa have led to a better understanding of the minimal machinery that is required for Fe–S cluster biogenesis. They have also indi-cated that there is an increased gene number and increased genetic complexity with increased organism complexity. The primitive parasitic prokaryotic cells of *Mycoplasma* spp. contain only IscS-, Nfu- and HscB-like homo-logues[66]. By contrast, no fewer than ten gene products that are involved in Fe–S cluster bio-genesis are present in numerous subcellular compartments in animals and plants. Genome analysis showed that *Arabidopsis thaliana* has three IscU homologues[22], and IscS homo-logues have been found in both chloroplasts and mitochondria[18]. The *A. thaliana* genome also encodes three chloroplast-localized and two mitochondria-localized Nfu homologues, and mutants that lack one of the chloroplast Nfu proteins showed a dwarf phenotype and decreased electron transport through photosystem I (REF. 22).

In mammalian cells, ISCS, ISCU and NFU have been identified in mitochondria, the cytosol and the nucleus. In all three cases, a single gene encodes different isoforms of each protein, which are targeted to different subcellular locations (FIG. 5). Mitochondrial and cytosolic/nuclear ISCS proteins are syn-thesized from a single transcript through initiation at alternative in-frame AUG codons. Initiation at the first AUG results in the inclusion of a mitochondrial targeting sequence in the protein, whereas initiation at the second AUG produces a protein that lacks a mitochondrial targeting sequence. Both ISCS isoforms contain a recognizable nuclear localization sequence[23]. Mitochondrial and cytosolic/nuclear ISCU proteins are generated through the selection of different exons in a single pre-mRNA[24]. Mitochondrial and cytosolic/nuclear NFU proteins are generated through the alternative use of 5′ splice donor sites[25]. So, the fundamental components that are needed for *de novo* Fe–S assembly in the cytosol are present, and recent functional studies have shown that they are involved in Fe–S cluster bio-genesis in mammalian cells (W.-H.T. and T.A.R., unpublished data).

In evolution, increased organismal com-plexity coincides with the demand for more elaborate transcriptional/translational con-trol mechanisms and greater diversity in the multisubunit complexes that facilitate Fe–S cluster assembly in different compartments. With the advance of genomics, it is increas-ingly evident that gene duplication, alterna-tive splicing and alternative transcription or translation initiation sites provide valuable evolutionary mechanisms for the creation of biological diversity[67,68]. Isoforms can be sorted to different subcellular sites, which provides a potential opportunity to control similar bio-logical processes in different cellular sites independently or in coordinated fashion. Potential crosstalk between mitochondria and the cytosol might function to regulate overall cellular iron uptake in response to increased use of iron by mitochondria.

Future prospects

The assembly of Fe–S clusters is a complex and important process for which the key proteins have been conserved throughout evolution. In recent years, important new roles for Fe–S proteins in cellular and mitochondrial iron homeostasis have been discovered. In Friedreich ataxia, the compromise of Fe–S cluster biogenesis leads to mitochondrial iron overload, which in turn leads to mito-chondrial oxidative damage and failure[40,49]. However, the mechanisms that underlie this iron overload are still unclear. Much of the efforts that have been aimed at treating patients with Friedreich ataxia have involved giving them antioxidants that might prevent the iron-dependent oxidative damage to lipids, DNA and proteins that occurs during the progression of the disease. If the connec-tion between impaired Fe–S assembly and mitochondrial iron overload could be fully elucidated, more direct treatments might be possible for Friedreich ataxia, as well as for some sideroblastic anaemias.
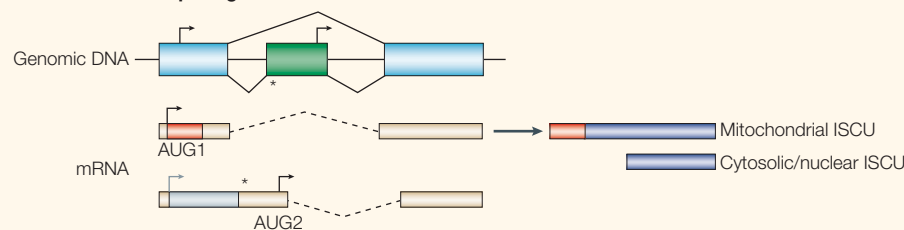
Tracey A. Rouault and Wing-Hang Tong are at the National Institute of Child Health and Human Development, Cell Biology and Metabolism Branch, Bethesda, Maryland 20892, USA.
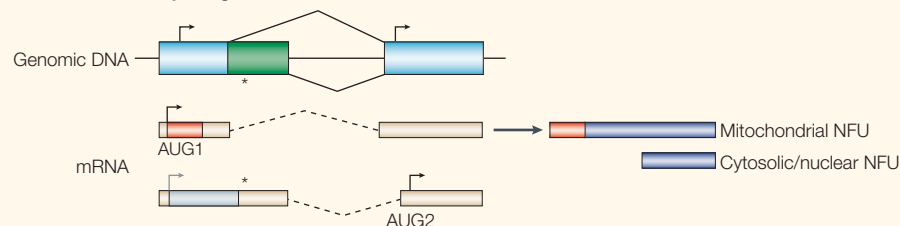*e-mails: trou@helix.nih.gov; tongw@mail.nih.gov*

ISCS: alternative use of in-frame start codons

ISCU: alternative splicing

NFU: alternative splicing

Figure 5 | **Isoforms of mammalian iron–sulphur (Fe–S) cluster assembly proteins.** Because of the sequence and functional similarities, mammalian Fe–S cluster assembly genes and proteins are shown here using the nomenclature that was originally proposed for the *Escherichia coli isc* operon. Through alternative splicing and the use of different translation-initiation sites, mammalian Fe–S cluster assembly genes generate mitochondrial and cytosolic/nuclear isoforms that differ at their N terminus. Alternative exons are shown as green boxes, mitochondrial targeting sequences are shown in red, upstream untranslated open reading frames are shown in grey, and in-frame stop codons are highlighted by asterisks.

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